Relationship between antioxidant potential and sodium pump activity in *Platorchestia platensis*

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Platorchestia platensis is a semi-terrestrial amphipod, often found as the dominant species in wrack beds [5]. In the wrack, the talitrid amphipod is exposed to varying levels of salinity. *Platorchestia platensis* and other talitrids are osmoregulators [4]. Any change in environmental salinity is likely to affect the animal's ATP demand for osmoregulation, and consequently, the production of reactive oxygen species (ROS) may vary. Although ROS are natural byproducts of oxidative metabolism, if unmatched by antioxidant defenses, they can cause significant damage to biological molecules (lipids, proteins, DNA) and macromolecular assemblages (biological membranes) [3]. Since changes in ATP demand with osmoregulatory challenges could significantly alter ROS production, we sought to determine if there exists a relationship between antioxidant and osmoregulatory capacities. Animals (*P. platensis*) were acclimated to different salinities and capacities for osmoregulation (Na^+/K^+-ATPase, NKA), oxidative metabolism (Cytochrome C Oxidase, CCO) and antioxidant defense (Trolox Equivalent Antioxidant Capacity, TEAC) were measured.

Platorchestia platensis was collected from Lab Beach at MDIBL. Animals were acclimated in the salinity environments for five days in 25% (278 $mOsm \cdot kg^{-1}$), 50% (470 $mOsm \cdot kg^{-1}$), and 100% (814 $mOsm \cdot kg^{-1}$) seawater. Different salinities were made up from ambient seawater diluted with distilled water. Twenty animals, 30 g of seaweed and 150 ml of water were placed in 1 L Nalgene plastic jars, with two jars per salinity, for a total of 6 jars. Ambient temperature (13.1°C) was maintained by floating the jars in filtered seawater in a cooler. Water inside the jars was replaced and osmolality checked daily. Whole animal samples were homogenized, and NKA [2] and CCO [7] were assayed at 20°C (controlled with a circulating water bath) in triplicate according to established procedures using a Pharmacia Ultraspec 3000. The improved ABTS cation decolorization assay was used to determine TEAC [6]. Values were normalized to protein content.

Table 1. Activities of Na^+/K^+ ATPase levels (NKA), Cytochrome C Oxidase levels (CCO), and Trolox Equivalent Antioxidant Capacity (TEAC) in *Platorchestia platensis*. Data are expressed as Units (U) per mg protein (U=µmol product/minute) for NKA and CCO. TEAC is expressed in µmol of Trolox Equivalent/mg protein. Data are means ± standard deviation (n=4).

	NKA (U/mg)	CCO (U/mg)	TEAC (µmol of Trolox Equivalent/mg)
25% seawater	0.010 ± 0.002	0.329 ± 0.063	0.282 ± 0.017
50% seawater	0.011 ± 0.004	0.354 ± 0.055	0.293 ± 0.015
100% seawater	0.011 ± 0.002	0.350 ± 0.051	0.287 ± 0.018

There were no significant differences ($p>0.05$) in NKA activity with salinity treatment (Table 1). Since NKA activity remained the same, it is not surprising that oxidative capacity (as indicated by CCO) and total antioxidant potential (as indicated by TEAC) were unaffected by salinity acclimation (Table 1). One explanation for the lack of change in NKA activity is that the seaweed may have provided a perch so that the animals were able to avoid immersion. Although our salinity acclimation was not sufficient to induce a change in NKA, there is a positive and statistically significant correlation between NKA activity and TEAC among all individuals sampled (Figure 1). These data suggest that as activities of NKA may rise, there is a concomitant increase in antioxidant potential. In order to test this

hypothesis adequately we will need to repeat the salinity acclimations in a way that ensures a change in NKA activity.

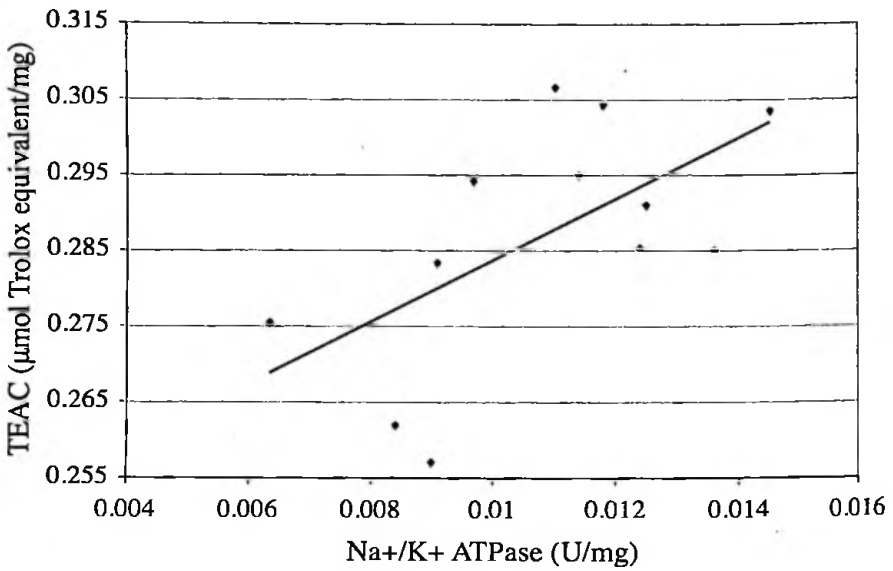

Fig. 1. TEAC correlates with activities of Na^+/K^+ ATPase (NKA) in *Platorchestia platensis*. Data represent activities measured in all individuals and are expressed as Units (U) per mg protein (U=µmol product/minute) for NKA. TEAC is expressed in µmol of Trolox Equivalent/mg protein. (r^2=0.36; p<0.05)

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1. **Abele, D., and S. Puntarulo.** Formation of reactive species and induction of antioxidant defence systems in polar and temperate marine invertebrates and fish. *Comparative Biochemistry and Physiology, Part A.* 138: 405-415, 2004.
2. **Barnett R.E.** Effect of monovalent cations on the ouabain inhibition of the sodium and potassium ion activated adenosine triphosphatase. *Biochemistry.* 9: 4644-4648, 1970.
3. **Halliwell, B. and J.M.C. Gutteridge.** *Free Radicals in Biology and Medicine 3rd Edition.* Oxford University, New York. 2004.
4. **Morritt, D. and A.M.M. Richardson.** Osmoregulation and Evolutionary Patterns in Coastal Terrestrial Amphipods (Talitridae). *Journal of Crustacean Biology.* 20: 67-74, 1999.
5. **Perrson, L.E.** Dispersal of *Platorchestia platensis* (Kröyer) (Amphipoda: Talitridae) along Swedish coasts: A slow but successful process. *Estuarine Coastal and Shelf Science.* 52: 201-210, 2000.
6. **Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans.** Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine.* 26: 1231-1237, 1999.
7. **Wharton D.C. and A. Tzagoloff.** Cytochrome oxidase from beef heart mitochondria. M.E. Pullman and R.W. Estabrook, eds. *Methods in Enzymology.* Academic Press, New York. 10: 245-250, 1967.